Acta Dermatovenerol APA

Acta Dermatovenerologica
Alpina, Pannonica et Adriatica

2025;34

Zebrafish as a new approach methodology (NAM) in dermatology: current research and the regulatory landscape focusing on sustainable development goals

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Supplementary Appendix 1 | Alignment of zebrafish new approach methodologies in dermatology research with sustainable development goals.

SDG goals	Zebrafish-based NAM contribution	Examples in dermatology	Relevance to LMICs
SDG 3 (good health and well-being)	Facilitates ethical, efficient, and affordable drug discovery and safety testing for skin diseases. Allows precision modeling of dermatological conditions.	Rapid screening of compounds for melanoma, psoriasis, vitiligo, eczema, wound healing, and genodermatoses. Identification of novel therapies for rare genetic skin diseases such as XMEA. Testing traditional formulations for efficacy and safety.	Allows the affordable and rapid screening of essential medicines and traditional formulations where clinical trials are limited. Supports the development of targeted treatments for prevalent skin conditions in LMICs.
SDG 9 (industry, innovation, and infrastructure)	Promotes biotechnological advancement through scalable, reproducible, and low-cost NAMs, replacing high-resource mammalian systems.	Integration into pharmaceutical and biotech dermatology pipelines for high-throughput screening of anti-inflammatory, anti-cancer, and pigmentation-modulating agents.	Encourages cost-effective research innovation in resource-constrained academic and public health institutions, fostering local research capacity and infrastructure development.
SDG 12 (responsible consumption and production)	Reduces the use of chemicals and animals in traditional testing, allowing micro-scale, low-waste research platforms.	Testing skin irritants, anti-aging creams, and pigmentation agents with fewer animal subjects and smaller chemical quantities.	Minimizes lab waste and animal use in LMICs with limited access to advanced waste disposal and ethical animal facilities, promoting resource efficiency.
SDG 13 (climate action)	Lowers the environmental footprint of dermatological research by reducing lab emissions and energy demands associated with large animal facilities.	Zebrafish-based toxicity assays instead of rodent models, leading to reduced energy consumption for animal housing and waste disposal.	An eco-friendly model that suits LMICs, which often face climate vulnerability and may lack sustainable laboratory infrastructure.
SDG 14 (life below water)	Helps assess the aquatic impact of dermatological products, preventing pollution of marine and freshwater ecosystems.	Monitoring the residues of skincare products, sunscreens, and their aquatic toxicity (e.g., impact of recycled plastics leachates on zebrafish lipid metabolism and endocrine systems).	Allows LMICs to test local product safety in aquatic environments without the need for expensive ecotoxicology infrastructure, protecting vital water resources.
SDG 17 (partnerships for the goals)	Enhances regulatory harmonization, knowledge exchange, and joint research efforts through NAM-focused consortia and open platforms.	Global NAM initiatives with OECD, WHO, EU Horizon, and NIH programs. Collaborative research projects on rare diseases or regional health challenges utilizing zebrafish models.	Promotes the inclusion of LMICs in global dermatology research networks and policy development for alternative test strategies, fostering equitable access to shared scientific knowledge and resources.

SDG = sustainable development goals, NAM = new approach methodologies, LMICs = low- and middle-income countries, XMEA = X-linked myopathy with excessive autophagy, OECD = Organisation for Economic Co-operation and Development, WHO = World Health Organization, EU = European Union, NIH = National Institutes of Health.

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Supplementary Appendix 2 | Application of zebrafish models for skin pigmentation disorders.

Model type	Study methodology	Observed effects (phenotype)	Key mechanisms
Physical induction method			
UVR method	Exposure to UVB (e.g., 300 mJ/cm² daily for 5 days or 8,100 mJ/cm² 5× daily)	Skin darkening, elevated melanin content	Triggers α-MSH/ACTH secretion, activates cAMP-PKA-CREB pathway, enhances melanin production
PEMFs	Exposure to pulsed electromagnetic fields (60 Hz, 2–20 G for 5–15 days)	Skin darkening, increased melanin granules	Upregulation of *DCT*, *TYRP-1*, *TYRP-2*, and *MC1R*; *MITF* activation; changes in ERK/p38 phosphorylation
Chemical induction method			
5-HT	Incubation in 5-hydroxytryptamine (0.01–1 mM)	Increased melanin content, darker skin stripes	Upregulates *MITF*, *TYR*, *TYRP-1*, and *TYRP-2*; activates PKA/p-CREB pathway
Fisetin	Exposure to fisetin (200–400 µM for 72 hours)	Enhanced melanin production, elevated *TYR* activity	Upregulates *MITF* and *TYR*; inhibits GSK-3β, leading to β-catenin accumulation and MITF activation
Flumequine	Exposure to flumequine (0–20 µM for 5–15 days)	Enhanced skin hyperpigmentation, increased melanin production	Upregulation of *TYR* and *MITF*; phosphorylation of p38, MAPK, and JNK
PPIX	Incubation with protoporphyrin IX (35–60 hours)	Increased body pigmentation, elevated *TYR* activity	Elevates cGMP and PKG activity, primarily through CREB signaling pathway
Ermanin	Incubation in ermanin-enriched medium	Enhanced melanin production, deeper skin pigmentation	Activates CREB-MITF pathway; upregulates *TYR*, *TYRP-1*, and *DCT*; alters GSH redox homeostasis; accumulates ROS
Complex mixtures	Exposure to EFE or RBM	EFE: enhanced *TYR* activity, melanin particle count; RBM: increased melanin content, gene expression	EFE: enhances *TYR* via MAPK/ERK1/2; RBM: downregulates ERK phosphorylation, activates ERK pathway
Genetic engineering			
Oncogenic *HRAS* expression	Crossing kita-GFP-RAS transgenic lines	Altered pigment patterns, heightened oxidation, surge in transformed melanocytes, melanoma formation	Oncogenic *HRAS* stimulates melanocyte growth/proliferation, hyperpigmentation contingent on copper uptake, p53 promotes melanoma
Xenograft human melanoma cells	Injection of human uveal melanoma cells into yolk sac of TG(fli1:EGFP) embryos	Excessive melanin deposition in eyes/skin, melanoma	Mutations in *GNA11/GNAQ* lead to MAPK pathway activation
bmp7b knockout	CRISPR-Cas9 construction of *bmp7b* mutant zebrafish	Increased retinal melanin, augmented skin melanin distribution, surge in body melanin content	Attributed to altered expression of *wnt7ba*, *gna14*, and *erbb3b*; counteracts *BMP7b's* natural inhibitory effect

UVR = ultraviolet radiation, UVB = ultraviolet B (280–315 nm), α-MSH = alpha-melanocyte-stimulating hormone, ACTH = adrenocorticotropic hormone, cAMP = cyclic adenosine monophosphate, PKA = protein kinase A, CREB = cAMP response element binding protein, PEMFs = pulsed electromagnetic fields, Hz = hertz; G = gauss, DCT = dopachrome tautomerase (TYRP-2), TYRP-1 = tyrosinase-related protein 1, TYRP-2 = tyrosinase-related protein 2, MC1R = melanocortin 1 receptor, MITF = microphthalmia-associated transcription factor, ERK = extracellular signal-regulated kinase, p38 MAPK = p38 mitogen-activated protein kinase, 5-HT = 5-hydroxytryptamine (serotonin), TYR = tyrosinase, p-CREB = phosphorylated cAMP response element binding protein, GSK-3β = glycogen synthase kinase-3 beta; β-catenin / beta-catenin, MAPK = mitogen-activated protein kinase, JNK = c-Jun N-terminal kinase, PPIX = protoporphyrin IX, cGMP = cyclic guanosine monophosphate, PKG = protein kinase G, GSH = glutathione, ROS = reactive oxygen species, EFE = Epimedii Folium extract, RBM = rice bran ash mineral extract, ERK1/2 = extracellular signal-regulated kinases 1 and 2, GFP = green fluorescent protein, HRAS = Harvey rat sarcoma viral oncogene homolog, kita = zebrafish ortholog of human *KIT* gene (stem cell factor receptor), p53 = tumor suppressor protein p53, GNA11 = guanine nucleotide-binding protein alpha 11, GNAQ = guanine nucleotide-binding protein alpha Q, TG(fli1:EGFP) = transgenic zebrafish line expressing enhanced green fluorescent protein under fli1 promoter (endothelial marker), bmp7b = bone morphogenetic protein 7b, CRISPR-Cas9 = clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9, wnt7ba = wingless-type MMTV integration site family member 7ba, gna14 = guanine nucleotide-binding protein alpha 14, erbb3b = erb-b2 receptor tyrosine kinase 3b.

Supplementary Appendix 3 | **Zebrafish models for genetic skin disorders.**

Human disease	Human gene and mutation	Zebrafish model	Methodology	Zebrafish phenotype
Epidermolysis bullosa (EB)				
EB simplex	*KRT5, KRT14*	Ortholog knockdown	Morpholino knockdown	Skin fragility, blistering, epidermal defects (inferred)
Dystrophic EB	*COL7A1*	Ortholog knockdown	Morpholino knockdown	Weakness in dermal–epidermal junction (inferred)
Ichthyosis				
Harlequin ichthyosis	*ABCA12*	*abca12* knockdown	Morpholino-based antisense oligonucleotides	Epidermal changes similar to human ichthyosis
CEDNIK syndrome	*SNAP29*	*snap29* knockdown	Morpholino-based antisense oligonucleotides	Epidermal changes similar to human ichthyosis
Pigmentation-related				
Human pigmentation polymorphisms	*SLC45A2*, SLC24A5	*slc45a2* mutant, *slc24a5* mutant	Humanized zebrafish orthologous rescue (HuZOR)	Rescue of embryonic mutant phenotypes, assessment of human mutation effects on gene function, influence on skin color

EB = epidermolysis bullosa, *KRT5* = keratin 5, *KRT14* = keratin 14, *COL7A1* = collagen type VII alpha 1 chain, *ABCA12* = ATP-binding cassette subfamily A member 12, *SNAP29* = synaptosomal-associated protein 29, *SLC45A2* = solute carrier family 45 member 2, *SLC24A5* = solute carrier family 24 member 5, HuZOR = humanized zebrafish orthologous rescue, *abca12* = zebrafish ortholog of human *ABCA12*, *snap29* = zebrafish ortholog of human *SNAP29*, *slc45a2* = zebrafish ortholog of human *SLC45A2*, *slc24a5* = zebrafish ortholog of human *SLC24A5*.

Supplementary Appendix 4 | **Key challenges and recommendations.**

Challenge	Description of challenge	Recommendations and ongoing efforts
Phylogenetic distance and limited translatability	Anatomical and physiological differences from mammals (e.g., non-keratinized skin and lack of sebaceous glands) reduce direct comparability of findings to humans.	Integrate zebrafish with complementary mammalian models, emphasize mechanistic and early-stage screening utility. Further comparative transcriptomic and proteomic studies to enhance correlation.
In-water dosing limitations	Reliance on water-soluble exposure limits applicability to hydrophobic drugs, difficulty correlating in-water exposure with mammalian plasma levels.	Develop advanced drug delivery methods (e.g., nanocarriers and encapsulation), validate alternative dosing routes, improve pharmacokinetic modeling tools.
Regenerative capacity	High regenerative ability (e.g., in retina, heart, and fin) may obscure chronic or irreversible toxicological endpoints.	Use mature or genetically altered zebrafish strains with reduced regenerative responses, focus on short-term outcomes or integrate with models lacking regenerative compensation.
Lack of standardization and reproducibility	Inconsistent husbandry, strain usage, and reporting practices hinder reproducibility across labs.	Promote harmonized protocols and reporting guidelines (e.g., ARRIVE for zebrafish), establish centralized zebrafish repositories and reference labs.
Cancer model–specific challenges	High variability in engraftment, immune rejection, yolk sac differences, and temperature mismatches in xenograft models.	Employ immunodeficient or casper lines for adult xenografts, optimize microinjection protocols, conduct comparative validation with murine models.
Fragmented regulatory framework	Absence of harmonized guidelines for dermatological endpoints limits regulatory acceptance and global data sharing.	Advocate OECD test guideline expansion to include dermatology-specific endpoints (e.g., pigmentation and wound healing), encourage regional regulatory agencies to adapt zebrafish NAMs through stakeholder engagement.
Limited capacity in LMICs	Lack of technical training and infrastructure restricts adoption of zebrafish NAMs in resource-limited settings.	Initiate training programs, equipment support, and North–South research partnerships to build zebrafish capacity in LMICs.
Translational uncertainty for dermatological use	Absence of benchmark protocols for skin-related assays (e.g., psoriasis and atopic dermatitis) delays uptake in drug development.	Fund targeted validation studies for inflammatory, pigmentation, and genodermatosis models; establish consensus dermatology-specific readouts.

ARRIVE = Animal Research: Reporting of In Vivo Experiments, OECD = Organisation for Economic Co-operation and Development, NAMs = New Approach Methodologies, LMICs = Low- and Middle-Income Countries.